

An Intramodular Thioesterase Domain Catalyses Chain Release in the Biosynthesis of a Cytotoxic Virulence Factor

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Supplementary Tables

Organism/pathway	Accession #	Class in SSN	Product	Domain arrangement for non-terminal TE domains
Burkholderia thailandensis TE-A	ABC36203	BurA TE-A and homologues	Gonyol	TE-A-T-KS-KR-TE-AT-ACP
<i>Micromonospora nigra</i> TE-A	WP_217628199	BurA TE-A and homologues		TE-A-T-KS-KR-TE-AT-ACP
<i>Pseudomonas</i> sp Irchel s3b6 TE-A	WP_095144283	BurA TE-A and homologues		TE-A-T-KS-KR-TE-AT-ACP
<i>Saccharothrix syringae</i> TE-A	WP_211269021	BurA TE-A and homologues		TE-A-T-KS-KR-TE-AT-ACP
<i>Streptomyces bryophytorum</i> TE-A	WP_240165141	BurA TE-A and homologues		TE-A-T-KS-KR-TE-AT-ACP
<i>Streptomyces glebosus</i> TE-A	WP_229894110	BurA TE-A and homologues		TE-A-T-KS-KR-TE-AT-ACP
<i>Streptomyces mobaraensis</i> TE-A	WP_152263742	BurA TE-A and homologues		TE-A-T-KS-KR-TE-AT-ACP
<i>Agrobacterium vitis</i> TE-B	WP_156583012	BurA TE-B and homologues		TE-A-T-KS-KR-TE-AT-ACP
Burkholderia thailandensis TE-B	ABC36203	BurA TE-B and homologues	Gonyol	TE-A-T-KS-KR-TE-AT-ACP
<i>Micromonospora nigra</i> TE-B	WP_217628199	BurA TE-B and homologues		TE-A-T-KS-KR-TE-AT-ACP
<i>Pseudomonas</i> sp Irchel s3b6 TE-B	WP_095144283	BurA TE-B and homologues		TE-A-T-KS-KR-TE-AT-ACP
<i>Saccharothrix syringae</i> TE-B	WP_211269021	BurA TE-B and homologues		TE-A-T-KS-KR-TE-AT-ACP
<i>Streptomyces bryophytorum</i> TE-B	WP_240165141	BurA TE-B and homologues		TE-A-T-KS-KR-TE-AT-ACP
<i>Streptomyces glebosus</i> TE-B	WP_229894110	BurA TE-B and homologues		TE-A-T-KS-KR-TE-AT-ACP
<i>Streptomyces mobaraensis</i> TE-B	WP_152263742	BurA TE-B and homologues		TE-A-T-KS-KR-TE-AT-ACP
DEBS_TEII <i>Saccharopolyspora erythraea</i>	CAA42028	Type II Thioesterase	P	
Megalomicin_TEII <i>Micromonospora megalomicea</i>	AAG13923	Type II Thioesterase	P	
Pikromycin_TEII <i>Streptomyces venezuelae</i>	AAC69333	Type II Thioesterase	P	
Borrelidin_TEII <i>Streptomyces</i> sp. CRB46	WP_019330222	Type II Thioesterase	P	
Tylosin_TEII <i>Streptomyces fradiae</i>	KDS84464	Type II Thioesterase	P	
Kendomycin_TEII <i>Streptomyces violaceoruber</i>	CAQ52621	Type II Thioesterase	P	
Rifamycin_TEII <i>Amycolatopsis mediterranei</i>	AAG52991	Type II Thioesterase	P	
Abyssomicin_TEII <i>Verrucosipora maris</i>	AEK75515	Type II Thioesterase	P	
Filipin_TEII <i>Streptomyces avermitilis</i>	BAC68117	Type II Thioesterase	P	
Kijanimicin_TEII <i>Actinomadura kijaniata</i>	ACB46473	Type II Thioesterase	P	
Maklamicin_TEII <i>Micromonospora</i> sp. GMKU326	BAQ25498	Type II Thioesterase	P	
Tetromadurin_TEII <i>Actinomadura verrucosopora</i>	QKG20162	Type II Thioesterase	P	
Tetronasin_TEII <i>Streptomyces longisporoflavus</i>	ACR50794	Type II Thioesterase	P	
Tetronomycin_TEII <i>Streptomyces</i> sp. NRRL 11266	BAE93720	Type II Thioesterase	P	
Thiolactomycin_TEII <i>Salinispora pacifica</i>	ALJ49917	Type II Thioesterase	P	
Gramicidin_TEII <i>Brevibacillus brevis</i>	BAH43865	Type II Thioesterase	N	

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Microcystin_TEII <i>Planktothrix agardhii</i>	ACA83967	Type II Thioesterase	N	
Versipelostatin_TEII <i>Streptomyces versipellis</i>	BAQ21958	Type II Thioesterase	P	
Tyrocidine_TEII <i>Brevibacillus brevis</i>	WP_0158910190	Type II Thioesterase	N	
Bacitracin_TEII <i>Bacillus licheniformis</i>	WP_020452080	Type II Thioesterase	N	
Naphthyridinomycin_TEII <i>Streptomyces lusitanus</i>	AGD80621	Type II Thioesterase	N	
Gramicidin_TEII_N_ <i>B.pseudomycooides</i>	WP_006096422	Type II Thioesterase	N	
Xenocoumacin <i>Xenorhabdus nematophila</i>	CBJ89763	Type II Thioesterase	PN	
Griseoviridin_TEII <i>Streptomyces griseoviridis</i>	AGN74877	Type II Thioesterase	PN	
Microtermolide_TEII <i>Streptomyces</i> sp. SID8354	WP_018540598	Type II Thioesterase	PN	
Spiruchostatin_TEII <i>Pseudomonas</i> sp. Q71576	AFR69340	Type II Thioesterase	PN	
Gramicidin_TEII <i>Bacillus pseudomycooides</i>	WP_006096422	Type II thioesterase	N	
Soraphen_TEI <i>Streptomyces</i> sp. Sp080513GE-23	AAA79984	Type I thioesterase domain	P	
Amphotericin_TEI <i>Sorangium cellulosum</i>	AAK73503	Type I thioesterase domain	P	
Lasalocid_TEI <i>Streptomyces lasalocidi</i>	BAG85032	Type I thioesterase domain	P	
Spinosyn_TEI <i>Saccharopolyspora spinosa</i>	AAG23262	Type I thioesterase domain	P	
Spirangien_TEI <i>Sorangium cellulosum</i>	CAD43451	Type I thioesterase domain	P	
Avermectin_TEI <i>Streptomyces avermitilis</i>	WP_010982381	Type I thioesterase domain	P	
Pikromycin_TEI <i>Streptomyces venezuelae</i>	AAC69332	Type I thioesterase domain	P	
DEBS_TEI <i>Saccharopolyspora erythraea</i>	WP_011873139	Type I thioesterase domain	P	
Meilingmycin_TEI <i>Streptomyces nanchangensi</i>	ADC45535	Type I thioesterase domain	P	
Lankamycin_TEI <i>Streptomyces rochei</i>	BAC76491	Type I thioesterase domain	P	
Curacin_A_TEI <i>Moorena producens</i> 19L	ACV42478	Type I thioesterase domain	P	
Mycinamicin_II_TEI <i>Micromonospora griseorubida</i>	BAC57032	Type I thioesterase domain	P	
Natamycin_TEI <i>Streptomyces chattanoogensis</i>	ADX66459	Type I thioesterase domain	P	
Aureothin_TEI <i>Streptomyces thioluteus</i>	CAE02606	Type I thioesterase domain	P	
Bacitracin_TEI <i>Bacillus licheniformis</i>	O68008	Type I thioesterase domain	N	
Lichenysin_TEI <i>Bacillus licheniformis</i>	CAA06325	Type I thioesterase domain	N	
Surfactin_TEI <i>Bacillus subtilis</i>	1JMK_C	Type I thioesterase domain	N	
Tyrocidine_TEI <i>Bacillus brevis</i>	O30409	Type I thioesterase domain	N	
Fengycin_TEI <i>Bacillus subtilis</i>	AAB00093	Type I thioesterase domain	N	
Mycosubtilin_TEI <i>Bacillus subtilis</i>	Q9R9I9	Type I thioesterase domain	N	
Bacillorin_TEI <i>Bacillus subtilis</i>	WP_007410142	Type I thioesterase domain	N	
Iturin_TEI <i>Bacillus subtilis</i>	ABY89500	Type I thioesterase domain	N	
Cephalosporin_C_TEI <i>Acremonium chrysogenum</i>	KFH48607	Type I thioesterase domain	N	
Isocomplestatin_TEI <i>Streptomyces lavendulae</i>	AAK81827	Type I thioesterase domain	N	

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Echinomycin_TEI <i>Streptomyces lasalocidi</i>	BAE98156	Type I thioesterase domain	N	
A54145_TEI <i>Streptomyces fradiae</i>	AAZ23078	Type I thioesterase domain	N	
Daptomycin_TEI <i>Streptomyces filamentosus</i>	AAX31559	Type I thioesterase domain	N	
Enduracidin_TEI-B <i>Streptomyces fungicidicus</i>	ABD65958	Type I thioesterase domain	N	C-A-T-C-A-T-C-A-T-C-A-T-C-A-T-C-A-T-TE-TE
Teixobactin TE1-B <i>Eleftheria terrae</i>	AJF34464	Type I thioesterase domain	N	C-A-T-C-C-A-T-C-A-T-C-A-T-C-A-T-TE-TE
Arthrofactin A TE1-B <i>Pseudomonas</i> sp. MIS38	BAC67536	Type I thioesterase domain	N	C-A-T-C-C-A-T-C-A-T-C-A-T-C-A-T-TE-TE
Massetolide A TE1-B <i>Pseudomonas fluorescens</i>	ABH06369	Type I thioesterase domain	N	C-A-T-C-A-T-C-A-T-TE-TE
Lysobactin TE1-B <i>Lysobacter</i> sp. ATCC 53042	AEH59100	Type I thioesterase domain	N	C-A-T-C-A-T-C-A-T-C-C-A-T-C-A-T-C-A-T-C-A-T-TE-TE
Syringopeptin_TEI-B <i>Pseudomonas syringae</i>	AAO72425	Type I thioesterase domain	N	C-A-T-C-A-T-C-A-T-C-A-T-C-A-T-C-A-T-C-A-T-C-A-T-C-A-T-C-A-T-TE-TE
Myxothiazol_TEI <i>Stigmatella aurantiaca</i> DW4/3-1	AAF19815	Type I thioesterase domain	PN	
Tubulysin_TEI <i>Archangium disciforme</i>	CAF05651	Type I thioesterase domain	PN	
Chondramid_TEI <i>Chondromyces crocatus</i>	Q0VZ70	Type I thioesterase domain	PN	
Cryptophycin_TEI <i>Nostoc</i> sp. ATCC 53789	ABM21572	Type I thioesterase domain	PN	
Hectochlorin_TEI <i>Lyngbya majuscula</i>	AAY42398	Type I thioesterase domain	PN	
Cylindrocyclophane_TEI <i>Cylindrospermum licheniforme</i>	AFV96142	Type I thioesterase domain	P TEII-like ¹	
Jerangolid_TEI <i>Sorangium cellulosum</i>	ABK32291	Type I thioesterase domain	P TEII-like ^{1,2}	
Ajudazol_TEI <i>Chondromyces crocatus</i>	CAQ18835	Type I thioesterase domain	PN TEII-like ³	
Legioliulin_TEI <i>Legionella parisiensis</i>	AIU36104	Type I thioesterase domain	P TEII-like ⁴	
Enduracidin_TEI-A <i>Streptomyces fungicidicus</i>	ABD65958	Other non-terminal thioesterase domain	N	C-A-T-C-A-T-C-A-T-C-A-T-C-A-T-C-A-T-C-A-T-C-A-T-C-A-T-TE-TE
Teixobactin TE1-A <i>Eleftheria terrae</i>	AJF34464	Other non-terminal thioesterase domain	N	C-A-T-C-C-A-T-C-A-T-C-A-T-C-A-T-TE-TE
Arthrofactin A TE1-A <i>Pseudomonas</i> sp. MIS38	BAC67536	Other non-terminal thioesterase domain	N	C-A-T-C-C-A-T-C-A-T-C-A-T-C-A-T-TE-TE
Massetolide A TE1-A <i>Pseudomonas fluorescens</i>	ABH06369	Other non-terminal thioesterase domain	N	C-A-T-C-A-T-C-A-T-TE-TE
Lysobactin TE1-A <i>Lysobacter</i> sp. ATCC 53042	AEH59100	Other non-terminal thioesterase domain	N	C-A-T-C-A-T-C-A-T-C-C-A-T-C-A-T-C-A-T-C-A-T-TE-TE
Syringopeptin_TEI-B <i>Pseudomonas syringae</i>	AAO72425	Other non-terminal thioesterase domain	N	C-A-T-C-A-T-C-A-T-C-A-T-C-A-T-C-A-T-C-A-T-C-A-T-C-A-T-C-A-T-TE-TE
Non-terminal TE1 <i>Mesorhizobium</i> sp. L48C026A00	WP_023803946	Other non-terminal thioesterase domain		TE-A-T-KS-AT-T
Non-terminal TE1 <i>Clostridium</i> sp. CAG:411	WP_022438322	Other non-terminal thioesterase domain		TE-A-KR-T-SDR
Non-terminal TE1 <i>Branchiostoma floridae</i>	XP_002598380	Other non-terminal thioesterase domain		KS-AT-DH-ER-KR-T-TE-C-A
Non-terminal TE1 <i>Mesorhizobium</i> sp. WSM3224	WP_027170255	Other non-terminal thioesterase domain		TE-A-T-KS-AT-T
Non-terminal TE1 <i>Coccomyxa subellipsoidea</i> C169	XP_005650993	Other non-terminal thioesterase domain		KS-MT-MT-MT-MT-T-T-T-T-KS-KR-T-T-KS-DH-ERKR-T-T-KS-KR-T-KS-KR-TT-T-KS-KR-T-T-KS-KR-TKS-DH-KR-T-T-KS-DH-KRT-T-KS-KR-T-TE-T-TE
Non-terminal TE1 <i>Mycobacterium abscessus</i>	WP_025239395	Other non-terminal thioesterase domain		TE-KS-AT-T
	XP_002905400	Other non-terminal thioesterase domain		TE-A-T-SDR

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FR901464 TE1 <i>Pseudomonas</i> sp. 2663	ADH01484	Other non-terminal thioesterase domain	P	DH-KR-AT-ACP-KS-KR-ACP-KS-ACP-ACP- TE -KS-ACP-C
Obafluorin_TEI <i>Pseudomonas fluorescens</i>	AQZ26587	Other non-terminal thioesterase domain	N	C-A-T- TE -A
FmoA5_TEI-1 <i>Streptomyces</i> sp. Sp080513GE-23	BAP16699	Other non-terminal thioesterase domain	N	C-T- TE -TE-A-T
FmoA5_TEI-2 <i>Streptomyces</i> sp. Sp080513GE-23	BAP16699	Other non-terminal thioesterase domain	N	C-T-TE- TE -A-T

Supplementary Table 1. Thioesterase sequences used to create a sequence similarity network. The thioesterase sequences were divided into five groupings: 1) BurA TE-A from *Burkholderia thailandensis* (in bold) and the equivalent TE-A domains from BurA homologues encoded in other bacteria (yellow); 2) BurA TE-B from *Burkholderia thailandensis* (in bold) and the equivalent TE-B domains from BurA homologues encoded in other bacteria (orange); 3) Type II thioesterases (teal); 4) Type I thioesterase domains (blue); and 5) Other non-terminal thioesterase domains (magenta). Where known, the product class (polyketide: P, non-ribosomal peptide: N, or polyketide-non-ribosomal peptide hybrid: NP) of the pathway that contains the thioesterase is included.

Primer name	Sequence 5'-3'. Restriction sites are underlined, mutated residues are highlighted using red bold.
S90A_OL1_Fw	GATTACATCTAGAAATAATTTTGTTAACITTAAGAAGG
S90A_OL1_Rv	CGTAGGCGAGCAGCGCGCCGAGGG GC GTGGCCGAACAGGAAGAAGGG
S90A_OL2_Fw	CCCTTCTCCTGTTTCGGCCAC GC CCTCGGCGCGCTGCTCGCTACG
S90A_OL2_Rv	GATTACAGAAATTCGCCGACGAGCGAATCGGGCCAGCTCTTTTCGAGC
S2023A_OL1_Fw	GCCCGATTCTGCTCGTCGGCGAATTCGTCGCGAGCTGCCGCTCGCGC
S2023A_OL1_Rv	CGCGGCCGCGCCCGGTAG GC CAGCCCGAGAACGCGACGGGCAGTTCCG
S2023A_OL2_Fw	CGAACTGCCGTCGCGTTCTGCGGGT GC CTACGCGCGCGCGCCGCG
S2023A_OL2_Rv	TCCTTCTGCGGCCGCAAGCTTCTACTCGTCCGCGATGCCGGCGACGT
S2749A_OL1_Fw	ACTGGCTGATCGACGAGCAGCAGGTGCCGCC
S2749A_OL1_Rv	GCGCGAGATCGAGCG C ATCGAGCCGAGCG
S2749A_OL2_Fw	CGCTCGGGCTCGAT GC GCTCGATCTCGCGC
S2749A_OL2_Rv	CGACGGCCGCCGACCACCGGTCCCG
pGEM_pbur_S90A_Up_Fw	TGAACCACCAAGTCTGTGGAGGTACCATGAGGCAAATAGTTTCAACG
pGEM_pbur_S90A_Up_Rv	CGTAGGCGAGCAGCGCGCCGAGGG GC GTGGCCGAACAGGAAGAAGGG
pGEM_pbur_S90A_Dn_Fw	CCCTTCTCCTGTTTCGGCCAC GC CCTCGGCGCGCTGCTCGCTACG
pGEM_pbur_S90A_Dn_Rv	AAGCTATGCATCCAACGCGTTGGGAGCTCTCCCATATGGTCGACCTGC
pET28a_TE-B_Fw	GATTACACATATGTCATGCAACGGCGGACATGA
pET28a_TE-B_Rv	ATATATATATAAAGCTTATCACAGCCACGCGGCCACGCGCGCGA
Tet_rv	CGGTGCCGAGGATGACGATG
II2087rvConf	CGAAGATCACGTCGTCGCCG

Supplementary Table 2. Oligonucleotide primers used in this study.

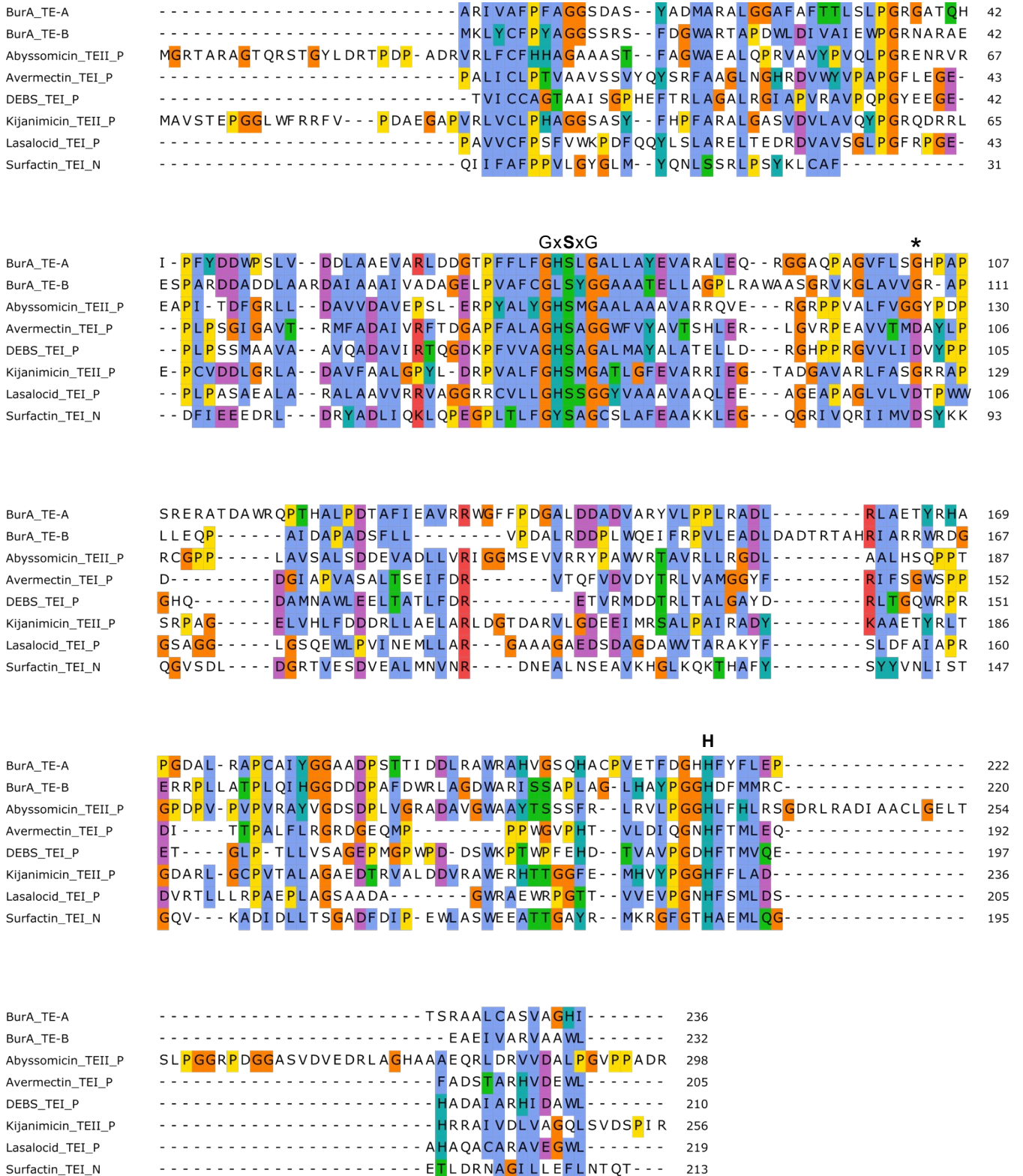
Plasmid	Description	Source
pHis8_svp	<i>E. coli</i> expression vector with His8-tag and svp; KanR	[5,6]
pHis8_svp_burA	pHis8-svp containing burA; KanR	[7]
pHis8_svp_burA_S90A	pHis8-svp containing burA S90A; KanR	This study
pHis8_svp_burA_S2023A	pHis8-svp containing burA S2023A; KanR	This study
pHis8_svp_burA_S2749A	pHis8-svp containing burA S2729A; KanR	This study
pET-28(+)	<i>E. coli</i> expression vector with a His6-tag; KanR	Novagen
pET-28(+) TE-B	<i>E. coli</i> expression vector containing a His6-tagged TE-B domain; KanR	This study
pET-28(+)TE-B S2023A	<i>E. coli</i> expression vector containing a His6-tagged TE-B S2023A domain; KanR	This study
pGEM_pbur	pGEM-easy vector for integrating PthaA promoter upstream of burA in <i>B. thailandensis</i> .	[8]
pGEM_pbur_S90A	pGEM-easy vector for integrating PthaA promoter upstream of burA and also introducing an S90A mutation in burA.	This study

Supplementary Table 3. Plasmids used in this study.

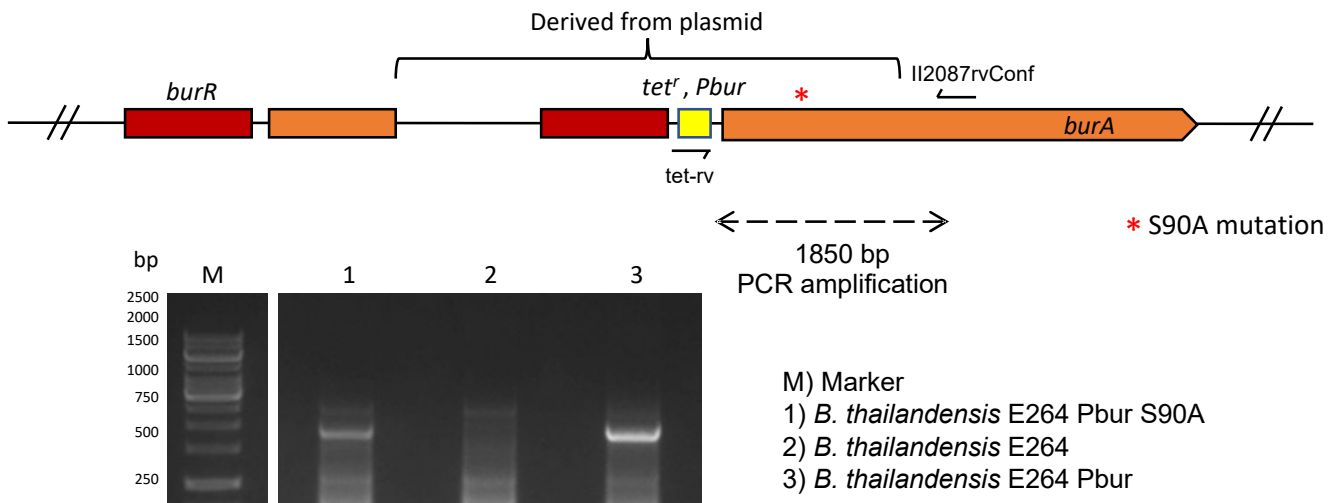
Name	Description	Source
<i>E. coli</i> TOP10	General cloning host strain	Invitrogen
<i>E. coli</i> Rosetta2 (λ DE3)	Protein expression host strain containing tRNAs for rare codons; CmR	Novagen
<i>E. coli</i> BL21 (λ DE3)	Protein expression host	New England Biolabs
<i>E. coli</i> BL21 (λ DE3) TE-B	Heterologous expression host for pET-28(+) TE-B	This study
<i>E. coli</i> BL21 (λ DE3) TE-B S2023A	Heterologous expression host for pET-28(+) TE-B S2023A	This study
<i>E. coli</i> Rosetta2 (λ DE3) pHis8_svp_burA_S90A	Heterologous expression host for pHis8_svp_burA_S90A	This study
<i>E. coli</i> Rosetta2 (λ DE3) pHis8_svp_burA_S2023A	Heterologous expression host for pHis8_svp_burA_S2023A	This study
<i>E. coli</i> Rosetta2 (λ DE3) pHis8_svp_burA_S2749A	Heterologous expression host for pHis8_svp_burA_S2749A	This study
<i>B. thailandensis</i> E264	Prototroph; environmental isolate	DSMZ
<i>B. thailandensis</i> E264 Pbur	The promotor of <i>burA</i> was exchanged with Δ PthaA;TetR	[8]
<i>B. thailandensis</i> E264 Pbur S90A	The promotor of <i>burA</i> was exchanged with Δ PthaA;TetR. An S90A mutation is present within <i>burA</i>	This study

Supplementary Table 4. Strains used in this study.

Supplementary Figures

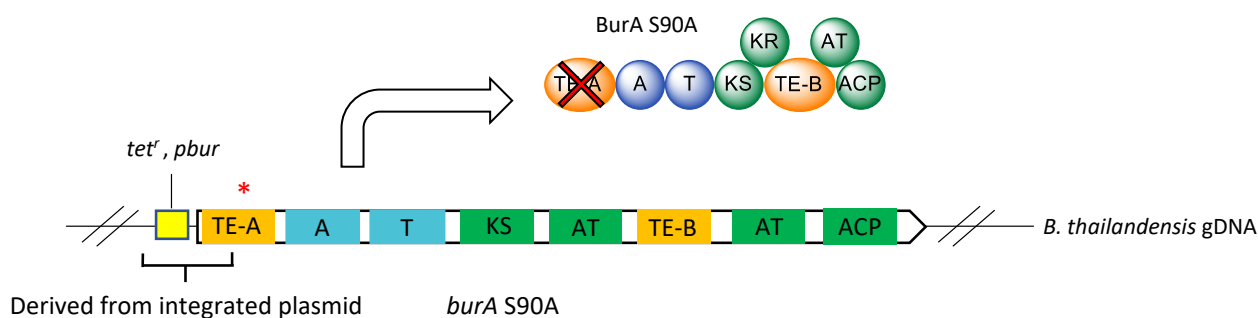


Supplementary Figure 1. Alignment of BurA TE-A, BurA TE-B, with other Type I thioesterase domains and Type II thioesterases. Alignment was made using MUSCLE⁹. The active site Ser and His residues are highlighted using the corresponding letter. The location of the conserved active site Asp is highlighted using an asterisk (*). Chemically similar or identical amino acids in an alignment are coloured accordingly: Blue, hydrophobic; Green, polar; Purple, negative; Red, positive; Yellow, proline; Orange, Gly.

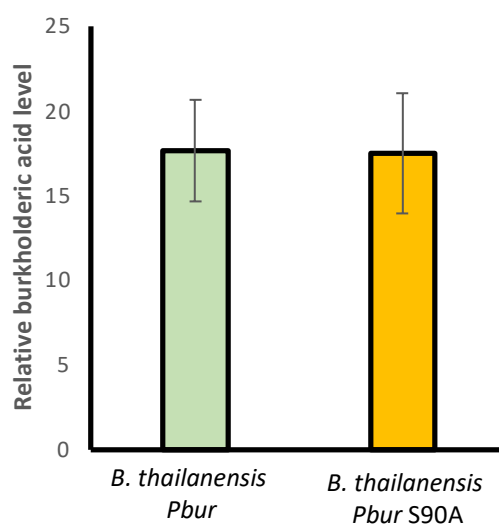


Supplementary Figure 2. Creation of a *B. thailandensis* E264 *burA* S90A mutant. The plasmid pGEM_*pbur_S90A* was transformed into *B. thailandensis* E264 by electroporation. Tetracycline resistant colonies were then screened using *tet-rv* and *I12087rv_Conf* primers (product size 1850 bp) to confirm that the desired plasmid integration had occurred, which simultaneously introduced the PTha⁸ promoter upstream of *burA* and introduced an S90A mutation into *burA*.

A

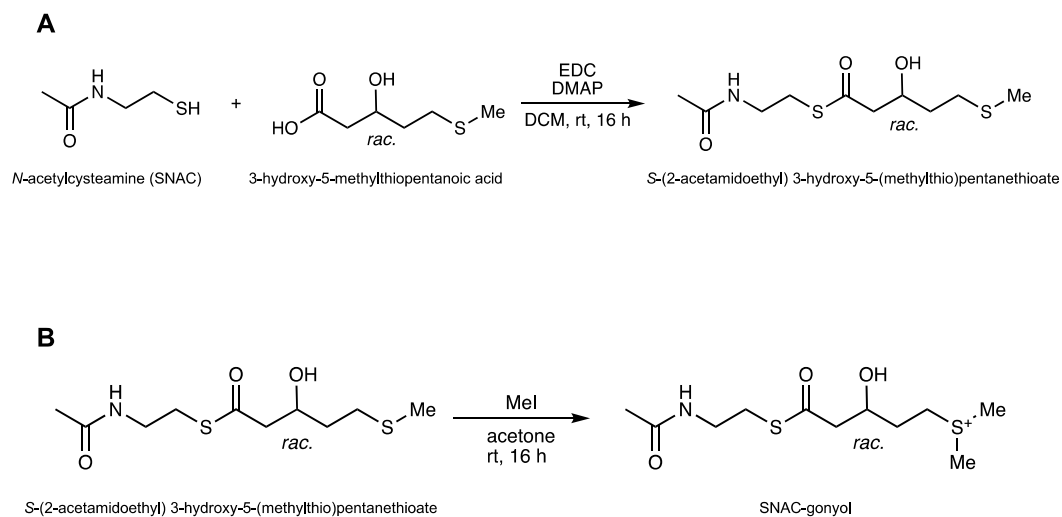


B

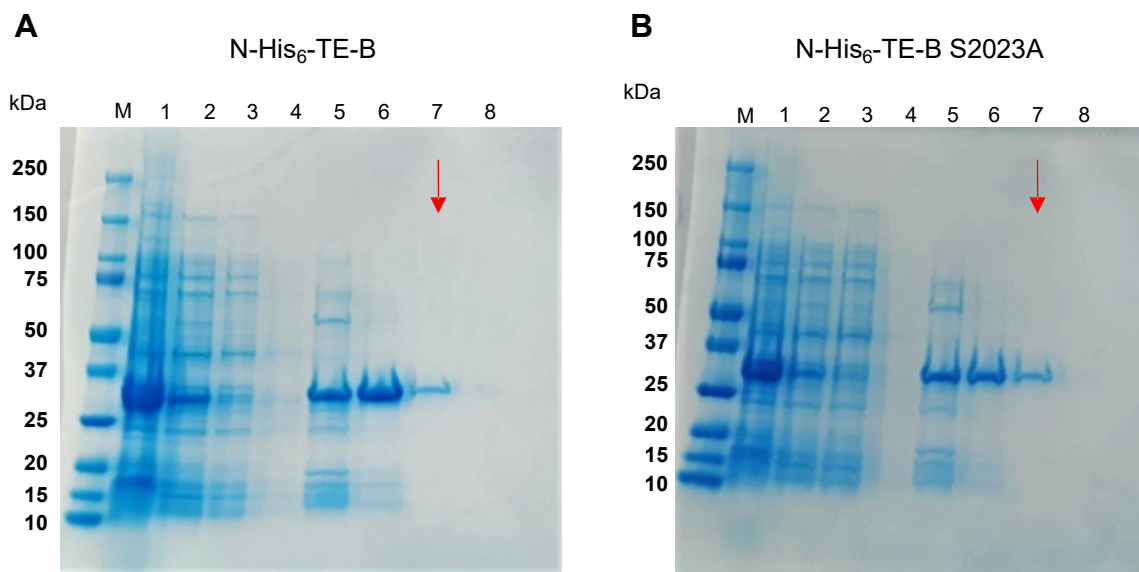


Sup

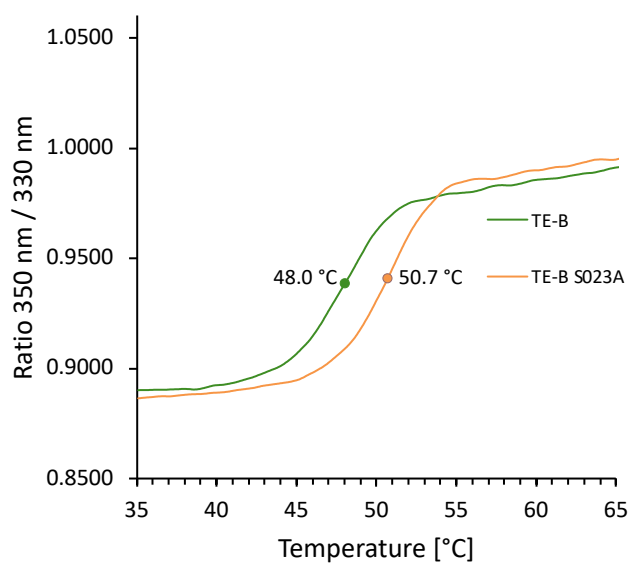
plementary Figure 3. Mutating TE-A in *B. thailandensis* E264. A) The desired S90A mutation in of *burA* TE-A was made in wild type *B. thailandensis* by transforming the plasmid pGEM_*pbur*_S90A. In addition to integrating a strong promoter upstream of *burA*, necessary for activating the *bur* cluster, pGEM_*pbur*_S90A also had a homology arm fragment to overwrite the 5' end of *burA* to add the S90A mutation in the gene region corresponding to TE-A. B) Levels of the highly UV absorbing burkholderic acid (Absorbance 370 nm) were unchanged in the *B. thailandensis* TE-A S90A mutant compared to the wild type. Burkholderic acid level is relative to a caffeine internal standard (Abs 270 nm). Data representative of three independent experiments.



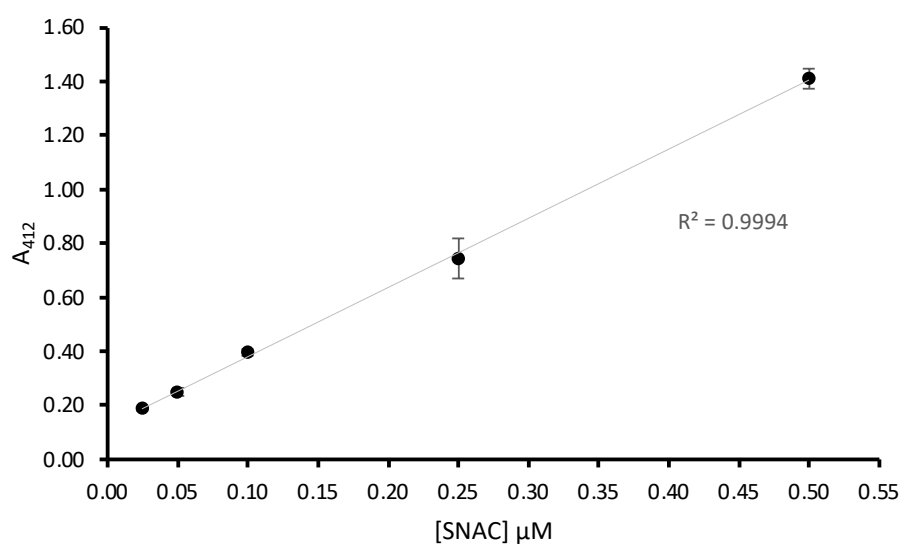
Supplementary Figure 4. A) Synthetic route to form racemic (2-acetamidoethyl) 3-hydroxy-5-(methylthio)pentanethioate, the precursor of racemic SNAC-gonyol. B) Synthetic route to form racemic SNAC-gonyol.



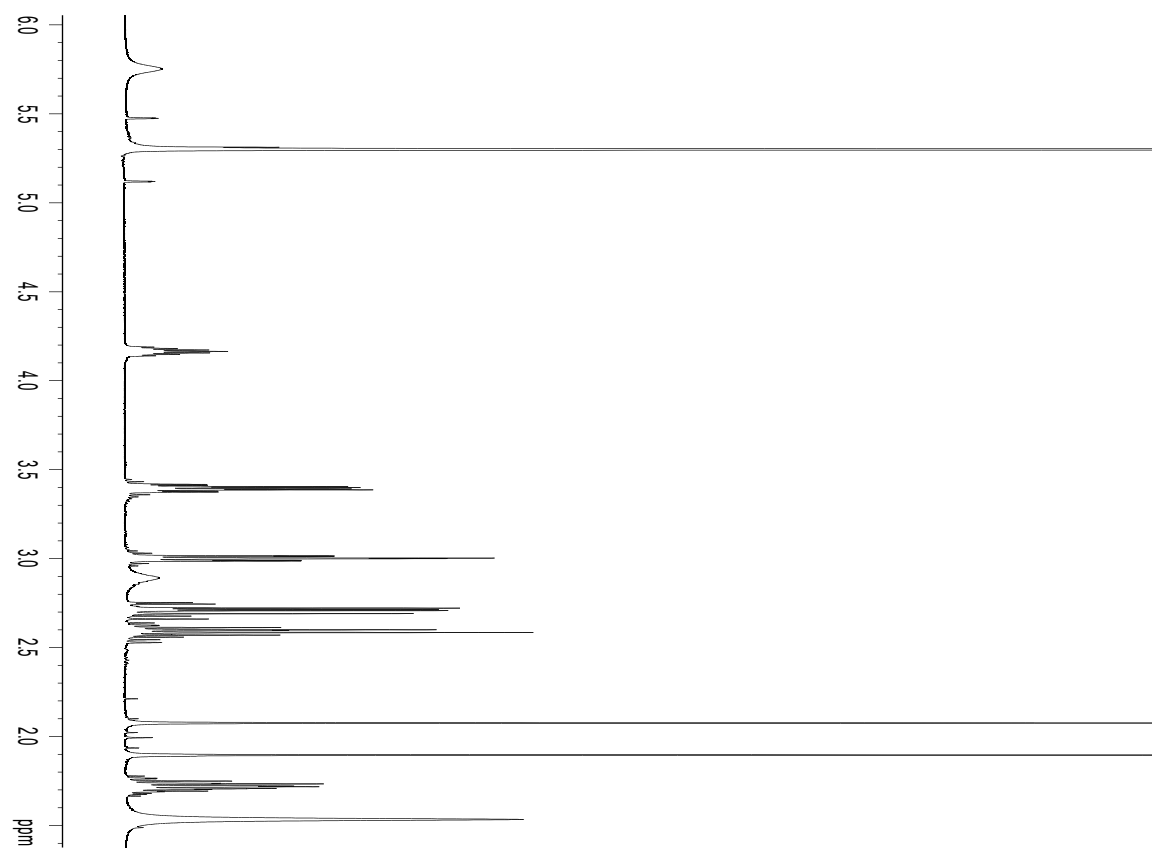
Supplementary Figure 5. Metal Affinity Purification of BurA TE-B and BurA TE-B S2023A. The gene region encoding **A.** the TE-B domain of BurA or **B.** were cloned into pET-28a in frame with an N-terminal His₆-tag. The TE-B protein was then purified using cobalt resin affinity chromatography. Each lane on the SDS-PAGE gel contains a different fraction from the purification. M) Protein marker ladder—Precision Plus Dual Colour (Bio-Rad). 1) Insoluble fraction. 2) soluble fraction. 3) Column flow-through. 4) Fraction from 20 mM TrisCl pH 7.9, 0.5 M NaCl, 10 % glycerol. 5) Fraction from 20 mM TrisCl pH 7.9, 0.5 M NaCl, 10 % glycerol, 50 mM imidazole. 6) Fraction from 20 mM TrisCl pH 7.9, 0.5 M NaCl, 10 % glycerol, 100 mM imidazole. 7) Fraction from 20 mM TrisCl pH 7.9, 0.5 M NaCl, 10 % glycerol, 200 mM imidazole. 8) Fraction from 20 mM TrisCl pH 7.9, 0.5 M NaCl, 10 % glycerol, 400 mM imidazole. The arrow highlights the lane corresponding to the elution fraction highly enriched for TE-B/TE-B S2023A. The theoretical mass of TE-B and TE-B S2023A is 31 kDa.



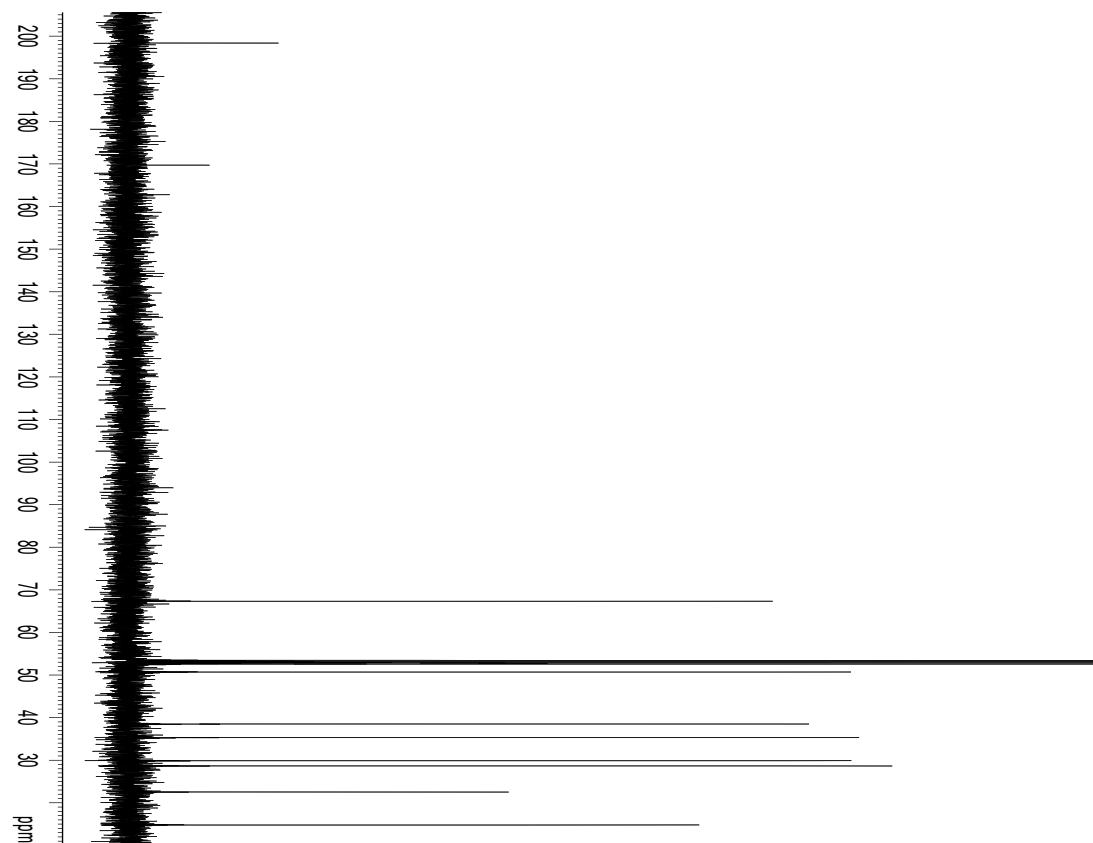
Supplementary Figure 6. The melting temperatures of purified TE-B and TE-B S2023A. The melting temperature of the mutant was very similar to the wild type.



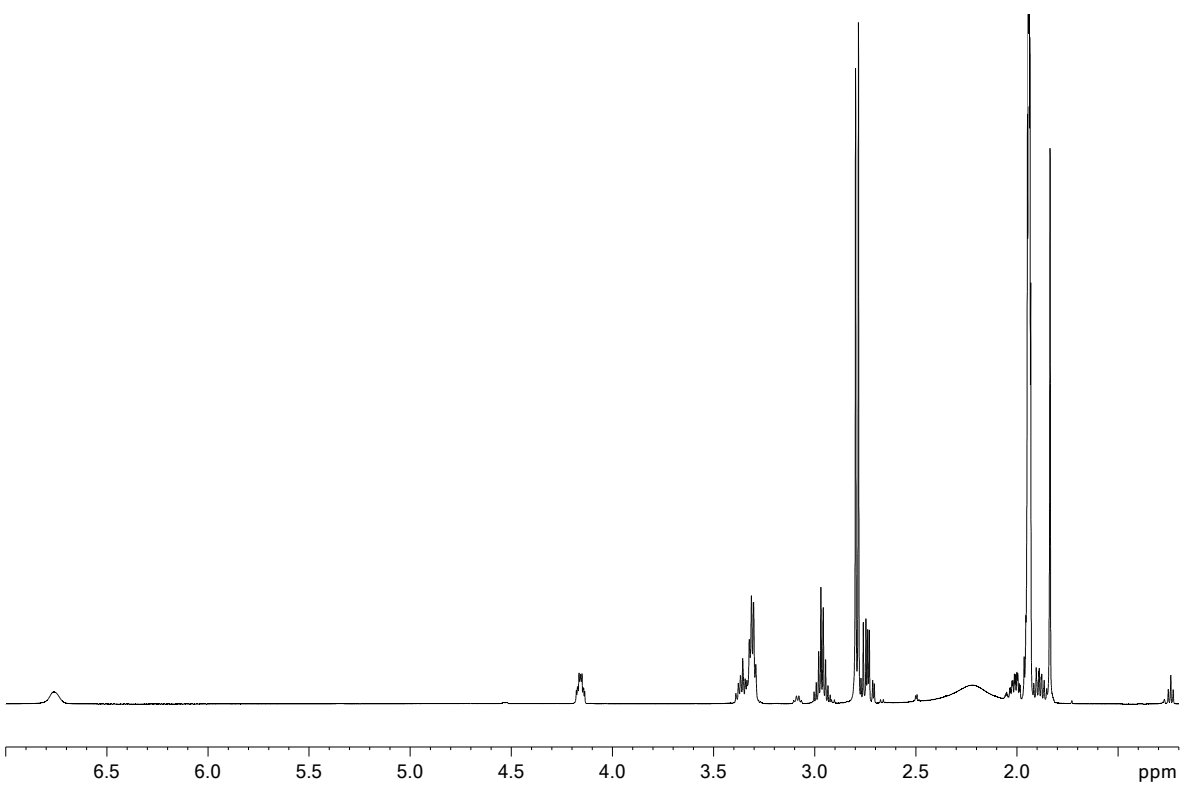
Supplementary Figure 7. SNAC standard curve used for the Ellman's reagent assay of TE-B. Different concentrations of SNAC were incubated in triplicate each with 10 μM (saturating) of Ellman's reagent, followed by measuring the absorbance at 412 nm.



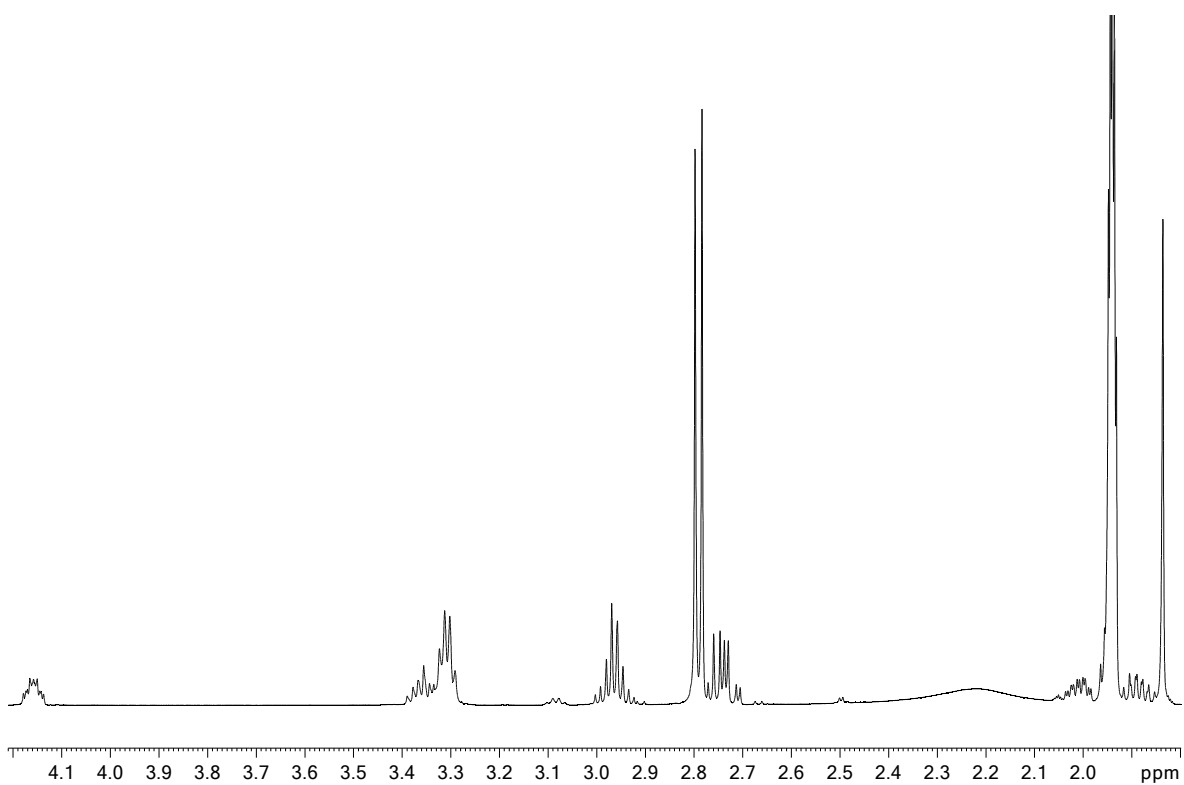
Supplementary Figure 8. ¹H NMR spectrum of racemic (2-acetamidoethyl) 3-hydroxy-5-(methylthio)pentanethioate (CD₂Cl₂).



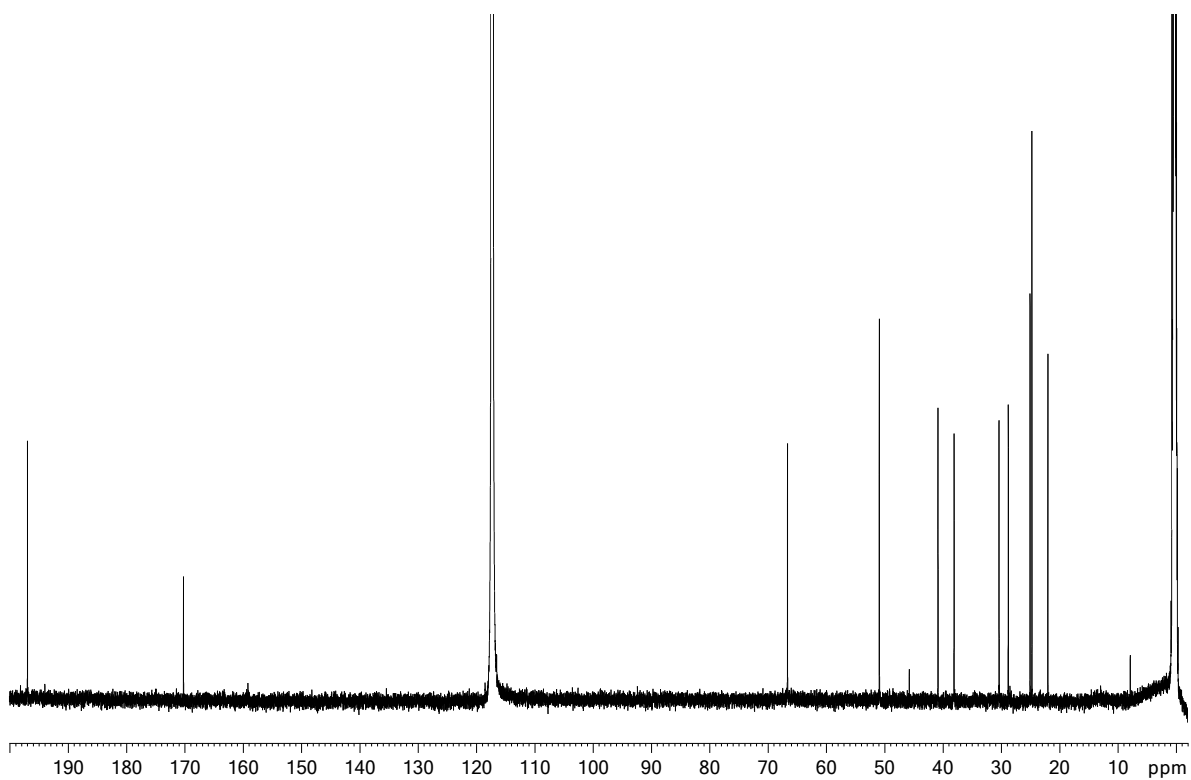
Supplementary Figure 9. ^{13}C NMR spectrum of racemic (2-acetamidoethyl) 3-hydroxy-5-(methylthio)pentanethioate (CD_2Cl_2).



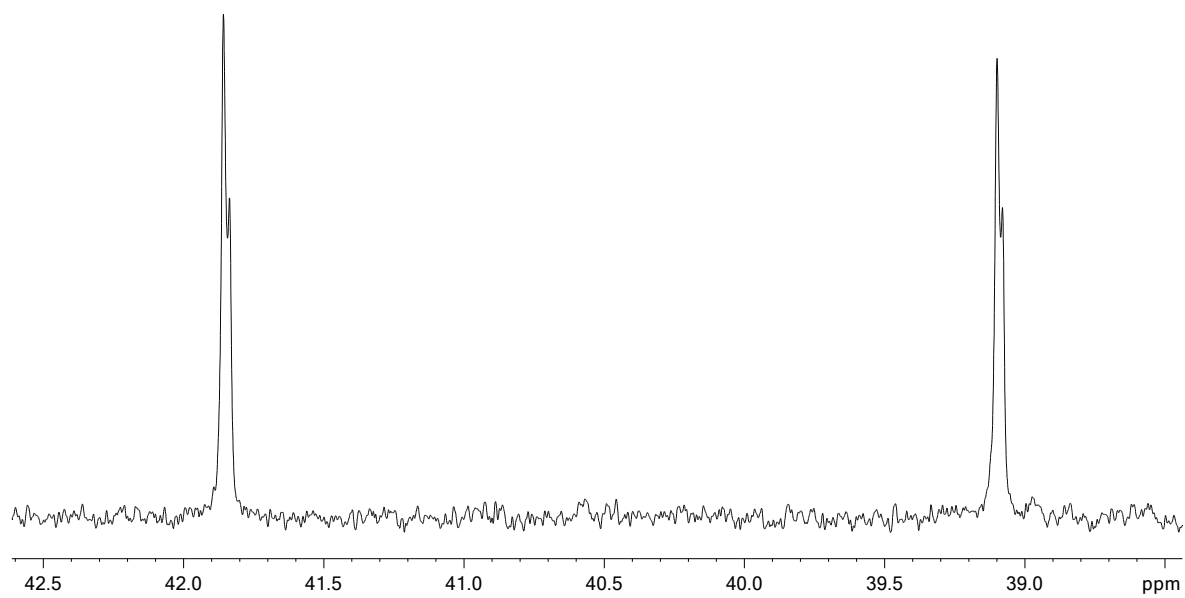
Supplementary Figure 10. Full ¹H NMR spectrum of racemic gonyol SNAC thioester (CD₃CN).



Supplementary Figure 11. Enlarged ^1H NMR spectrum of racemic gonyol SNAC thioester (CD_3CN).



Supplementary Figure 12. Full ^{13}C NMR spectrum of racemic gonyol SNAC thioester (CD_3CN).



Supplementary Figure 13. Enlarged ^{13}C NMR spectrum of racemic gonyol SNAC thioester (CD_3CN).

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